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14. ABSTRACT:

ONE OF THE MAJOR LIMITATIONS FACING THE THERAPEUTIC USE OF ONCOLYTIC VIRUSES, INCLUDING ONCOLYTIC HSV, IS THAT THE PRE-EXISTING ANTI-VECTOR IMMUNITY CAN SUBSTANTIALLY REDUCE THE INFECTIVITY OF THE VIRUS. WE PROPOSED IN THE AIM 3 OF THIS FUNDED PROJECT TO ADDRESS THIS ISSUE WITH TWO STRATEGIES: 1) TO DELIVER ONCOLYTIC HSVS THROUGH LIPOSOME-FORMULATED VIRAL DNA INSTEAD OF THE TRADITIONAL VIRAL PARTICLES, AND 2) TO USE T-LYMPHOCYTES AS A CARRIER FOR ONCOLYTIC HSV DELIVERY. IN TESTING THE FIRST STRATEGY, OUR DATA DEMONSTRATED THAT VIRAL DNA FORMULATED WITH LIPOSOMES GAVE THE HIGHEST NUMBER OF PLAQUES. VIRAL CAPSIDS FORMULATED WITH LIPOSOME GAVE THE SECOND BEST RESULT. IN TESTING THE SECOND STRATEGY, OUR RESULTS SHOWED THAT ONCOLYTIC HSV COULD BE EFFICIENTLY LOADED ONTO THE CARRIER CELLS USING A "HITCHHIKE" METHOD AND WAS THEN SUCCESSFULLY "HANDED OFF" TO THE TARGET CELLS. WE CONCLUDE THAT THIS "HITCHHIKE" STRATEGY REPRESENTS A VERY EFFICIENT WAY OF LOADING ONCOLYTIC VIRUS TO THE CARRIER CELLS, WHICH ARE OTHERWISE NONPERMISSIVENESS TO INFECTION OF ONCOLYTIC HSVS.

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INTRODUCTION

One of the major limitations facing the therapeutic use of oncolytic viruses, including oncolytic HSV, is that the pre-existing anti-vector immunity can substantially reduce the infectivity of the virus (1,2). This is especially problematic when the therapeutic application requires repeated and systemic delivery (3), such as in the case of treating metastatic prostate cancer. A variety of strategies have been reported to circumvent the anti-viral immunity for repeated viral vector administration (4-7). However, only limited success has been achieved so far.

We proposed in the Aim 3 of this funded project to address this issue with two strategies. For the first strategy, we proposed to deliver oncolytic HSVs through liposome-formulated viral DNA instead of the traditional viral particles. Although only a small percentage of tumor cells might be initially transduced by the liposome formulated HSV DNA, the subsequent virus replication in the initially transduced cells would produce hundreds or even thousands of progeny, which can then infect surrounding cells. For the second strategy, we also proposed to use T-lymphocytes as a carrier for oncolytic HSV delivery. T lymphocytes can freely circulate through the vascular system and can infiltrate to the metastatic tumors. Furthermore, the cell membrane can function as a protective shield to protect the virus from neutralizing antibodies.

BODY

We initiated the experiments to test both strategies. However, due to some personnel changes, we have not finished the entire project yet. Following are the key preliminary data we have collected. We anticipate that we will finish these experiments within the one year of no-cost-extension.

Liposome formulation of oncolytic HSVs. To test the condition of liposome formulation of oncolytic HSVs, we prepared the virus in 3 different forms: 1) DNA form of viral genome; 2) intact viral particles;

3) de-enveloped viral capsid. We then formulated these different forms of virus preparations with liposomes and examined their infectivity in vitro by counting the plaques after adding them onto Vero cell monolayers. The results showed that viral DNA formulated with liposomes gave the highest number of plaques. Viral capsids formulated with liposome gave the second best result. The intact viral particles formulated with liposome produced the lowest number of viral plaques, possibly because the intact viral particles already contain an outside lipid membrane, which may have prevented liposome formulation.

Delivery of oncolytic HSVs by carrier cells. We initially tested the infectivity of oncolytic HSVs on T lymphocytes. The cells were infected with Baco-1, which carries the green fluorescent protein (GFP) marker gene in its viral genome and its infectivity can thus be easily identified. The results showed either human or murine T lymphocytes were resistant to infection by oncolytic HSVs. Even at an multiplicity (MOI) of 10, less than 5% of cells showed GFP expression. We then examined NK cells and macrophages, another two cell types that are major components of circulating blood. In addition, both NK cells and macrophages have the ability to infiltrate to tumor tissues. We initially performed a similar in vitro experiment as described above to test the infectivity of the oncolytic Baco-1 on these cells. The results showed that both NK cells and macrophages from human and murine origins were also resistant to infection by Baco-1. At an MOI of 5, less than 10% of cells showed GFP expression.

Recently it has been reported that retrovirus can adhere nonspecifically, or 'hitchhike', to the surface of cytotoxic t lymphocytes (CTLs). CTLs hitchhiked with a retroviral vector can then move to tumor sites and “hand off” the viruses to tumor cells to initiate virus infection (8). We thus tested this strategy to determine if oncolytic HSV could hitchhike to carrier cells that were found to be nonpermissive to infection of HSV vectors in our previous experimnets. We incubated either NK cells or macrophages of both human and murine origins ($1X10^5$) with $5X10^5$ plaque-forming-units (pfu) of oncolytic HSV

Baco-1 for 1 h. The cells were then gently washed 2 times with PBS and were added to Vero cell monolayers. Viral plaques were examined 48 h later. The results showed that Baco-1 was efficiently loaded to the carrier cells in this way and was then successfully “handed off” to the testing cell monolayers. Since more than $1X10^5$ plaques were obtained from each preparation (with $1X10^5$ carrier cells), it was concluded that approximately 100% of the carrier cells were able to hand off the oncolytic virus. These results indicate that this hitchhike strategy may be a useful way for carrier cell-medicated delivery of oncolytic HSVs for the treatment of metastatic prostate cancer.

KEY RESEARCH ACCOMPLISHMENTS

The results obtained from the in vitro studies demonstrated that:

- Preparation of oncolytic HSV in the form of either DNA or de-enveloped capsid could be more efficiently formulated with liposomes than the intact viral particles. Thus, these two forms of viral preparation will be used in the future for in vivo studies.
- NK cells and macrophages could also function as carrier cells for delivery of oncolytic HSVs.
- The “Hitchhike” strategy represents a very efficient way of loading oncolytic virus to the carrier cells, which are otherwise nonpermissiveness to infection of oncolytic HSVs.

REPORTABLE OUTCOMES

- On March 2, 2006, Dr. Zhang was invited to give a seminar presentation to the clinical oncologists in the Texas Children’s Hospital. Title of presentation: A novel oncolytic virus for therapy of solid tumors.

CONCLUSIONS

Progresses have been made on both strategies designed to develop alternative ways to deliver oncolytic HSVs to metastatic prostate cancer. Especially for the second strategy, i.e., using immune cells as carriers for virus delivery, our data demonstrate that the recently reported “hitchhike” strategy is an ideal way of attaching oncolytic HSVs to carrier cells that are otherwise non-infectable by HSV, thus overcoming a major hurdle of this project. We are confident that the remaining tasks of this project will be finished during the one-year no-cost-extension period.

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APPENDICES:

None